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Enzymatic Bromo-ether Cyclization of Laurediols with Bromoperoxidase

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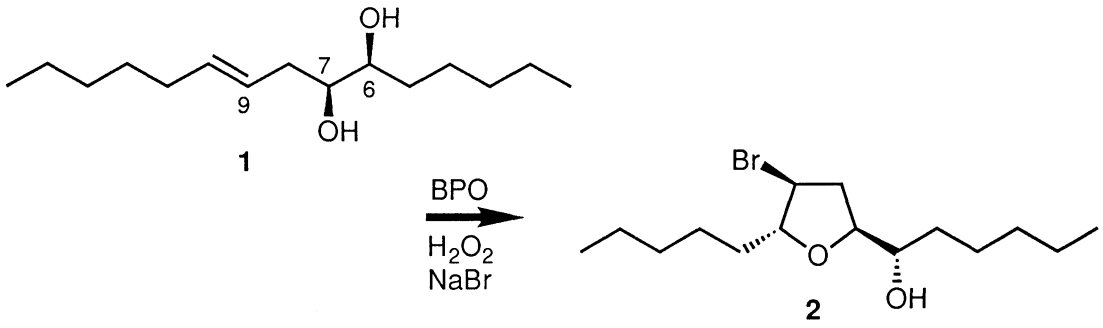
Natural (3*E*,6*R*,7*R*)- and (3*Z*,6*S*,7*S*)-laurediols were subjected to enzymatic reaction with the partially purified bromoperoxidase from the red alga, *Laurencia nipponica*, in the presence of H_2O_2 and NaBr to afford laurencin and prelaureatin, respectively. The further reaction of these products with bromoperoxidase gave rise to the bicyclic bromo-ethers.

Various 8-membered cyclic ether compounds having bromine atom have been isolated from the red algae, *Laurencia* species.[1,2] These compounds have been presumed to be produced biogenetically from linear-chained laurediols[3] by intramolecular cyclization by bromoperoxidase (BPO). Recently, we have reported such cyclization of laurediols by using lactoperoxidase (LPO), which is similar in character to BPO.[4] In this paper, we describe the partial purification of BPO from the red alga, *Laurencia nipponica*, and several enzymatic reactions of laurediols by the BPO.

The red alga, *L. nipponica*, (3 kg wet weight) collected at the west coast of Oshoro Bay, Hokkaido Island, on June, was ground together with dry ice, homogenized in 0.1 M (=0.1 mol dm^{-3}) potassium phosphate buffer (pH 5.5, 1.5 l) for 20 min, and filtered through cheesecloth. The filtrate was centrifuged (3000 rpm) at 2 °C for 1 h. The clear supernatant was regarded as the the primary enzyme extracts. The extracts were purified further by a modification of the Hager's procedure;[5] to the solution was added slowly solid $(NH_4)_2SO_4$ (201 g) with stirring at 0 °C in order to bring the extracts to 25% $(NH_4)_2SO_4$ saturation. The solution, which was kept at 5 °C overnight, was centrifuged at 10000 rpm for 1 h to afford the supernatant and pellets. The supernatant was brought to 65% saturation by addition of solid $(NH_4)_2SO_4$ (316 g) at 0 °C, and stirred at 5 °C overnght. Centrifugation of the suspension at 9000 rpm gave the pellets, which were again dissolved in cold 0.2 M Tris buffer (pH 7.0, 100 ml) and centrifuged at 10000 rpm at 2 °C for 1 h yielding the supernatant. This was applied to DEAE-Sephadex column (4 cm x 45 cm) and eluted with 0.2 M Tris buffer (1 l) to remove pigment fractions at 2 °C overnight, though it was difficult to remove completely the pigments characteristic of red algae. Finally, the active BPO fraction was eluted from the column with 0.2 M~1.0 M Tris buffer (pH 7.0) as a linear gradient (the flow rate was maintained at 7.5 ml/h and 72 fractions consisting of 8.0 ml each were collected). The BPO activity of the fractions thus isolated was measured optically in the decrease of absorbance at 278 nm on reaction with chlorodimedone in the presence of H_2O_2 and KBr in the phosphate buffer (pH 6.8) and the specific activity of the best fraction was fifty folds higher than that of the primary extracts of the red alga. Furthermore, the enzyme was found to contain an iron porphyrin similar to LPO judging from the

decrease of the activity by the inhibitors such as KCN or NaN_3.

First of all, (6*S*,7*S*,9*E*)-9-pentadecene-6,7-diol **1** was employed as the substrate of the model experiment (Scheme 1). A solution of **1** (27.3 mg, 5.4 mM) in DMSO (0.2 ml) was added into the solution of NaBr (4.8 mM) in phosphate buffer (pH 5.5, 50 mM, 20 ml). To the mixture was added an aliquot of each solution consisting of 0.1 M H_2O_2 (168 μl, final concentration 0.8 mM) and the BPO (600 μl) each in 12 portions during 2 h and the mixture was allowed to react at 23 °C for 24 h. The extracts were chromatographed over SiO_2 and purified by HPLC to provide an oxolane compound **2** (0.5 mg, 1.4%) along with a mixture of bromohydrins (2.0 mg, 5.2%) and the recovered starting material (22.0 mg). Compound **2** was identical with an authentic sample[6] in ^{1}H-NMR spectrum. Next, we carried out the reaction under the same conditions as above *without BPO* and found that *no reaction occurred.* The results reveal that compound **2** was produced only in the presence of BPO. Accordingly, we could regard the above reaction involving formation of **2** as *the enzymatic reaction.*

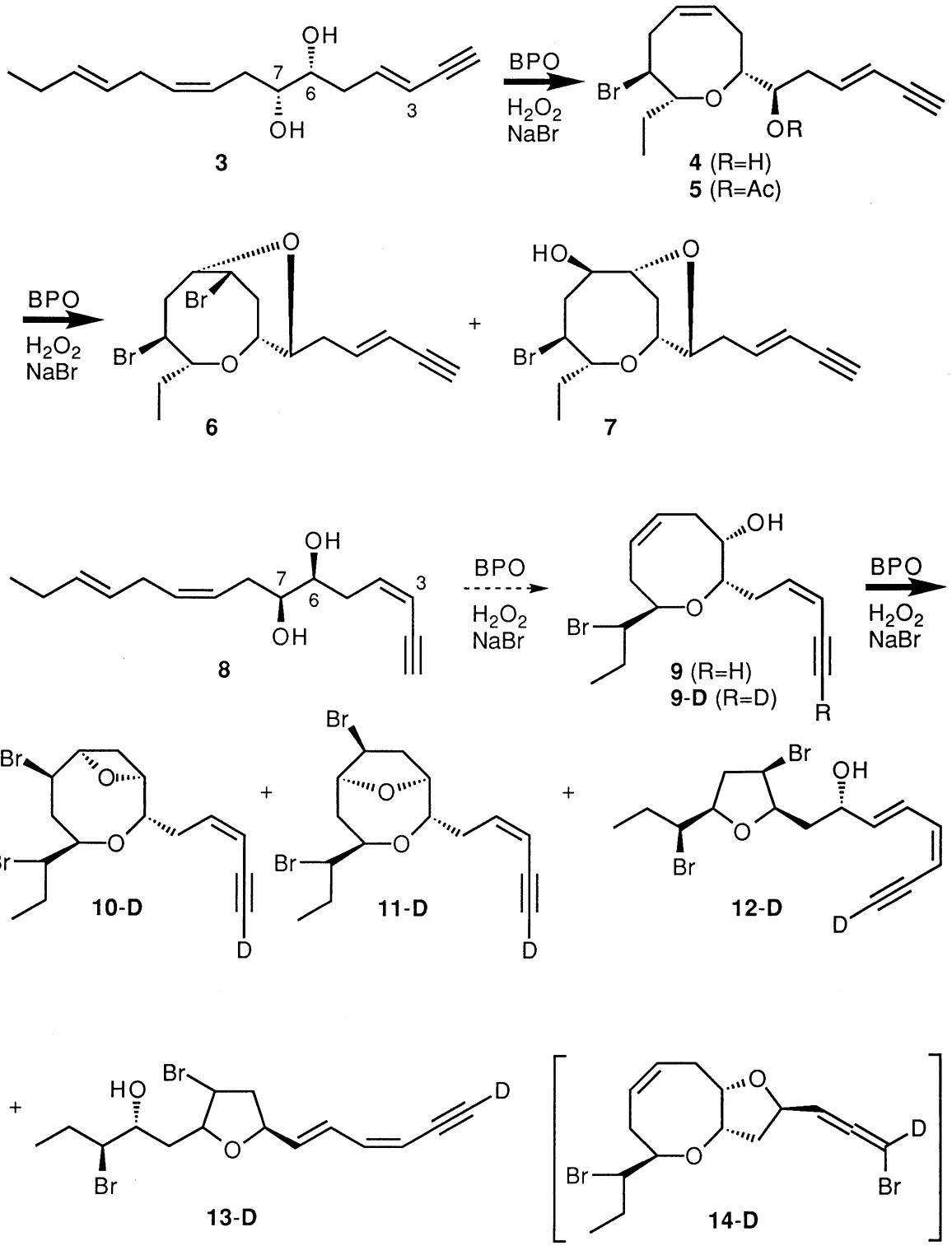


Scheme 1.

On the basis of the above model experiment, we extended the reaction to use (3*E*,6*R*,7*R*)-laurediol **3** as the substrate (Scheme 2). To a solution of **3** (101.0 mg, 10.6 mM) and NaBr (10.0 mM) in a 1:100 mixture (40.5 ml) of DMSO and phosphate buffer (pH 5.5), were injected aliquots of each solution of H_2O_2 (totally 2.0 mM) and the BPO (2.0 ml) by dividing into 12 portions under an atomosphere of argon at 5 °C during 2 h. The mixture was stirred at 5 °C for 24 h. The extracts was separated on SiO_2 column to give rise to deacetyllaurencin **4** (0.02 mg, 0.015%), an unknown cyclic bromo-ether (0.1 mg, 0.074%), bromohydrins (3.2 mg, 2.24%), and the recovered starting **3** (83.2 mg, 82.4%). Deacetyllaurencin **4** was identified after acetylation with natural laurencin **5** in respects of HPLC (Radial Pak μ-Porasil with hexane-CH_2Cl_2-CH_3CN (50:49:1) and ^{1}H-NMR spectrum. These results reveal that *BPO is the real enzyme for the direct bromo-ether cyclization of* **3** *to* **4**. This constitutes the first direct evidence for the possible biosynthetic route to laurencin skeleton. Furthermore, deacetyllaurencin **4** was allowed to react with the BPO in the presence of H_2O_2 and NaBr at 20 °C for 24 h to afford laureoxanyne **6** (0.8%), laurefucin **7** (1.3%), bromohydrins (12.6%), and the recovered starting **4** (72.2%). Compounds **6** and **7** were identical with respective authentic samples with regards to ^{1}H-NMR spectra. These results were analogous to the cases of the enzymatic reactions with LPO.[4]

Next, we attempted the enzymatic reaction with (3*Z*,6*S*,7*S*)-laurediol **8** as the substrate (Scheme 2). The reaction was carried out in a similar way to above at 0 °C for 24 h. The reaction afforded prelaureatin **7** (a trace amount) as well as a mixture of bromohydrins (8.0%) and the recovered starting **8** (82.0%). Although we could not measure any spectral data of the fraction **9** for identification, its retention time on HPLC [Develosil

60-3 with hexane-CH_2Cl_2-CH_3CN (70:20:10)] corresponded exactly to the authentic sample of **9**. Finally, [1-^{2}H]-prelaureatin **9-D** was subjected to the enzymatic reaction (0 °C → 10 °C, 24 h) and yielded [1-^{2}H]-laureatin **10-D** (0.07%), [1-^{2}H]-isolaureatin **11-D** (0.05%), and two bromo-oxolanes **12-D** (0.9%), **13-D** (0.3%), along



Scheme 2.

with bromohydrins (5.7%) and the recovered starting **9-D** (69.0%). Compounds **10-D** and **11-D** were identified by comparison with authentic samples by ^{1}H-NMR and the rention time on HPLC (Develosil 60-3 with hexane-CH_2Cl_2-CH_3CN (80:19:1)], respectively. The structures of **10-D** and **11-D** were tentatively elucidated by ^{1}H-NMR spectral data.[7] It is to be noted that [1-^{2}H]-laurallene **14-D** could not be detected even in a trace amount on HPLC in contrast with the case of LPO.[4]

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7) **12-D**; ^{1}H-NMR (400 MHz, $CDCl_3$), δ6.81 (1H, ddt, J=11.0, 15.4, and 1.1 Hz), 6.45 (1H, t, J=11.0 Hz), 5.92 (1H, dd, J=6.2 and 15.4 Hz), 5.45 (1H, d, J=11.0 Hz), 4.46 (2H, m), 4.13 (2H, m), 3.92 (1H, dt, J=8.8 and 3.7 Hz), 2.90 (1H, ddd, J=6.6, 7.7, and 15.0 Hz), 2.61 (1H, ddd, J=1.8, 4.4, and 15.0 Hz), 2.16 (1H, ddq, J=2.5, 14.6, and 7.3 Hz), 2.05 (1H, ddd, J=5.5, 8.8, and 14.3 Hz), 1.91 (1H, dt, J=3.7 and 14.3 Hz), 1.75 (1H, m), and 1.08 (3H, t, J=7.3 Hz). **13-D**; ^{1}H-NMR (400 MHz), $CDCl_3$), δ6.84 (1H, ddt, J=11.0 and 15.0 Hz), 6.47 (1H, t, J=11.0 Hz), 5.93 (1H, dd, J=6.5 and 15.0 Hz), 5.47 (1H, d, J=11.0 Hz), 4.44 (1H, br q, J=6.5 Hz), 4.22 (2H, m), 3.72 (2H, m), 2.72 (1H, dt, J=11.3 and 4.0 Hz), 2.47 (1H, m), 2.26 (2H, m), 2.12 (1H, ddd, J=2.5, 7.3, and 14.6 Hz), 2.02 (1H, m), and 0.98 (3H, t, J=7.3 Hz).

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